plates. Gels without Hap were used as controls. The gels were kept in culture for 3 weeks in αMEM medium containing 10%FCS. Gel contraction and cell viability, morphology and metabolism were analyzed.
Results: Gels, attached to surface plates after fabrication, became free-floating gels contracted from day 3, only in the presence of cells. A linear rapid contraction phase was observed until day 7, then a very slow contraction phase took place. The incorporation of Hap improved gel stability as compared to collagen gels without Hap.
Total DNA increased two times from day 3 to day 21, reflecting cell proliferation inside gels. Moreover, while cell metabolism increased all over the culture period, an important increase was observed after 3 weeks. Cells cultured in gels showed elongated spindle-shape morphology and a few dead cells were observed all over the experiment. Microscopy images showed that cells were randomly distributed on the gel. After proliferation cells were connected, mainly at the gel surface, which could stabilize collagen contraction.
Conclusions: This study shows the feasibility and biocompatibility of hydroxyapatite supplemented collagen gels for the culture of mesenchymal stem cells. They could be used as carriers for cell delivery in osteoarticular regenerative medicine.

532

PRENATAL DEVELOPMENTAL HEALING WITH INTRA-ARTICULAR GROWTH HORMONE (IAGH) INJECTIONS TREATS A VARIETY OF OSTEOARTHRITIC AND RHEUMATOID JOINTS WITH A SUCCESS RATE OF 75% TO 95% AND NO COMPLICATIONS

A.R. Dunn
Mt. Sinai Med. Ctr., Miami Beach, FL

Purpose: To utilize the prenatal developmental scar-free healing promoted by intra-articular growth hormone (IAGH) injections. The basic science demonstrates that pre-natal developmental healing (PDH) occurs without bleeding, clot formation and the in-pouring of inflammatory cells and fibroblasts, the cascade of post-natal healing. PDH produces real articular cartilage and no fibrocartilage. A study of 66 patients with advanced osteoarthritis of the knee treated with IAGH resulted in 87 per cent good and excellent results and the patients did not need total knee arthroplasty (TKA). In light of these results the author has treated many other joints with equally good to excellent results. Methodology including pre-treatment testing and results are here reported.
Methods: Every patient was evaluated before treatment with blood tests, X-rays and MRI's. Weight had to be within one standard deviation of normal.
Patients were instructed in exercising and use of crutches during treatment. Patients agreed in writing to the requirements for treatment. Many hip, knee, and ankle patients required arthroscopic debridement and abrasion chondroplasty prior to receing IAGH injections. Joint were injected with a range of dosage of recombinant Human Growth Hormone which ranged from 2.5 mgm to 10 mgm per injection depending on the size of the joint. Injections were given weekly for 3 to 15 weeks depending on the response which was determined by bi-weekly X-rays. Patients were allowed to drop out of the program at will: none dropped out.
Results: The success rate was as follows: Ankles 95%; knees 87%; hips 60%; subtalar and talonavicular joints 95%; shoulders 75%; elbows 95%; wrists 95%; base of thumb 75%. There were no complications, allergic reactions, infections, deep vein thrombosis, pulmonary embolism, or deaths from the IAGH treatments of over 800 patients.
Conclusions: An alternative treatment to total joint arthroplasty and other treatments is presented. It utilizes the ability of Growth Hormone injections to provide Pre-natal Developmental Healing. The advantages of PDH are: ease of application; lack of complications, side-effects and infection; and high patient satisfaction. The cost of treating hips, knees, and ankles is 1/4 that of joint replacement arthroplasty which can cost US$35,000.00 for each joint, without complications. If complications occur with joint arthroplasties the cost can easily double. Revision arthroplasty also increase the costs of total joints. Patients treated with IAGH may need booster injections once every one to five years and the cost is small.
The author recommends that IAGH treatment be used before indicating total joints because if the IAGH treatment is not effective in a small percentage there are no down-sides. No joints have become worse from IAGH treatment.
In summary, a treatment which is effective, safe, and economical is presented as alternative to joint replacements.

Therapy – Intraarticular

533

Therapeutic EFFECTS OF INTRA-ARTICULAR ADMINISTRATION OF ULTRA-PURIFIED LOW ENDOTOXIN ALGINATE ON THE PROGRESSION OF EXPERIMENTAL OSTEOARTHRITIS IN RABBITS

T. Igarashi, N. Iwasaki, D. Kawamura, N. Tsukuda, A. Minami
Hokkaido Univ., Sapporo, Japan

Purpose: Recent clinical studies have demonstrated no therapeutic effects of HA on preventing the OA progression. We developed highly purified biocompatible alginate, which can drastically reduce the endotoxin level. Our hypothesis was that the ultra-purified low endotoxin alginate (UPLE-alginate) could promote anti-arthritic activity in experimental OA. The aims of this study were to clarify the effect of the UPLE-alginate administration on OA progression and on joint lubrication, and to determine the adequate molecular weight of the UPLE-alginate using a rabbit OA model.
Methods: Three UPLE-alginates were prepared: AL20 (MW 0.43×10^6 Da), AL100 (1.0×10^6 Da), and AL500 (1.70×10^6 Da) (Mochida Pharmaceutical Co., Ltd., Tokyo, Japan). For comparison, sodium HA (ARTZ®, Kaken Pharmaceutical Co., Ltd., Tokyo, Japan) were used. *OA model and treatment protocols.* Japanese white rabbits (2.8-3.0 kg) were used according to the established ethical guidelines approved by the local animal care committee. To induce OA, ACL transection (ACLT) was performed. All knees were randomly divided into 5 groups as follows; AL20, AL100, AL500, HA, and normal saline (NS) groups. Intra-articular injections of a 0.3 ml of each material (1%) started at 4 weeks for a total of 5 weekly injections. All animals were euthanized at 9 weeks.
Assessments. Gross morphologic findings were assessed according to criteria by Yoshioka et al (n=10 knees). For histological evaluation, the sections were stained with safranin-O. Cartilage degeneration was quantitatively analyzed using the scoring system described by Kikuchi (n=8 knees). Six knees in each group were prepared for mechanical testing. The effect of the intraarticular injection on joint lubrication was assessed using a pendulum friction tester designed by our laboratory for small samples. The friction coefficient was calculated from the obtained data.
Statistical analysis. Significant differences among the groups were assessed by a two-way ANOVA followed by multiple-comparison post hoc tests. Two-tailed *P* values less than 0.05 were considered statistically significant.
Results: *Gross Morphology.* The NS and HA groups showed extensive cartilage erosion mainly at the medial femoral condyle. The alginate injection groups exhibited milder degradation than the HA and NS groups. The AL100 group showed lower severity grades. Severe OA changes of grade 3 (overt fibrillation) and grade 4 (erosion) at the medial femoral condyle

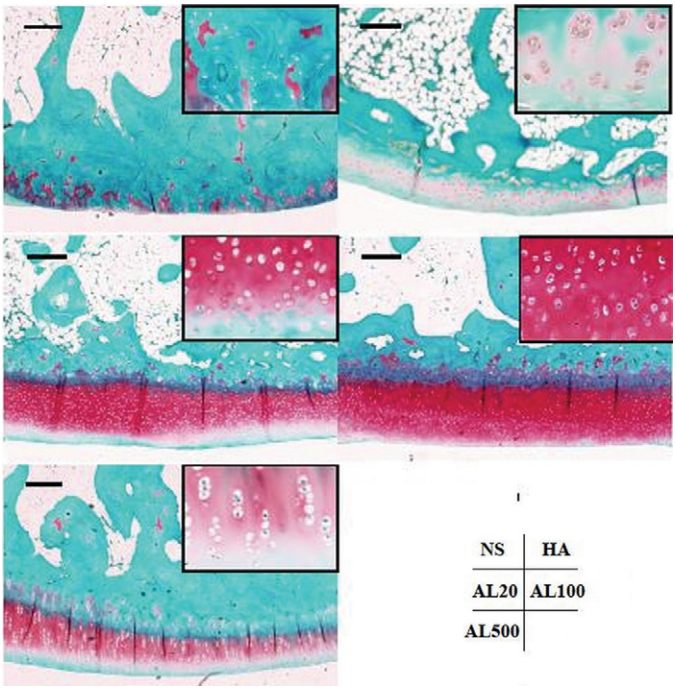

Figure 1

occurred at a rate of 60%, 63%, 67%, 70%, and 90% in AL100, AL20, AL500, HA, and NS, respectively.
Histology. In the NS group, loss of the superficial layer, fibrillation, and cleft were observed. In the HA and the AL500 groups, cartilage degeneration including fibrillation, fissures, and loss of proteoglycan was observed in the femoral condyle. An obvious reduction in the severity was found in the AL100 group. The overall degenerative score of the AL100 group tended to be the lowest, indicating that AL100 suppressed degradation. The overall scores of the treatment groups were significantly lower than that of the NS group (AL20, AL100, and AL500 vs NS, $p<0.01$; HA vs NS, $p<0.05$).
Friction. The friction coefficients of the AL100 and the AL20 group were significantly lower than that of the NS and the HA group ($P<0.05$).

Table 1. Histological scores and friction coefficient

	Histological score	Friction coefficient
NS	21.3±1.3	0.0294±0.0014
HA	17.3±1.2	0.0235±0.0013
AL20	14.9±0.9	0.0148±0.0027
AL100	12.6±0.9	0.0122±0.0037
AL500	15.4±0.9	0.0232±0.0117

Conclusions: The current study is the first to examine the influence of alginate materials on OA progression in vivo. Our findings suggest that intraarticular administration of the UPLE-alginates is effective in preventing articular cartilage degeneration and on improving joint lubrication of OA knees induced by ACLT. In terms of molecular weight dependency, AL100 (1.0×10^6 Da) has more therapeutic effects on OA progression. Based on these results, we reasonably conclude that the UPLE-alginates, especially AL100, have promising potential for becoming an effective agent of intraarticular injection for preventing OA progression.

534

EFFECTS OF INTRA-ARTICULAR INJECTIONS OF HYLAN GF-20 ON SERUM AND URINE BIOMARKERS IN PATIENTS WITH KNEE OSTEOARTHRITIS: THE BIOVISCO STUDY

T. Conrozier[1], X. Chevalier[2], J.-C. Balblanc[3], P. Richette[4], F. Rannou[5], D. Mulleman[6], B. Maillet[7], J.-F. Maillefert[8], P. Mathieu[1], Y. Henrotin[9], E. Vignon[1]
[1]centre Hosp. Lyon Sud, Pierre Bénite, France; [2]Hôpital Henri Mondor, Créteil, France; [3]centre Hosp. de Belfort, Pierre Bénite, France; [4]Hôpital Lariboisiere, Paris, France; [5]Hôpital Cochin, Paris, France; [6]Hôpital Trousseau, Tours, France; [7]Clinique Sainte Odilon, Moulins, France; [8]centre Hosp. de Dijon, Dijon, France; [9]Hôpital Universitaire, Liège, Belgium

Purpose: Viscosupplementation (VS) by intra articular (IA) injection of hyaluronic acid (HA) is widely used to reduce pain in patients with knee osteoarthritis (K-OA). However little is known on its effect on joint metabolism as well as on its possible structure modifying effect. Objectives: To investigate the effect of VS on circulating OA biomarkers in patients with K-OA.
Methods: Prospective open label study. 51 patients with unilateral symptomatic K-OA (ACR criteria; Kellgren-Lawrence grade I to IV) received an IA injection of 2mL of HA (hylan GF-20) IA injection on days (D) 1, 7, 14 and were followed 3 months. At D-15 patients were examined and X-rays were performed, in order to exclude patients with bilateral K-OA, or those with more than 3 OA joints including the target knee. From D-15 to D90 concomitant therapies were unchanged. Walking pain (WP) on VAS was obtained at each visit. Clinical response was defined as a WP decrease >30 mm between D1 and D90 (50% improvement). Urine (U) and serum (S) samples were obtained, using a standardized procedure, 2 weeks before the first injection (D-15), then at D1 (1st injection), D30 and D90. S-C2C, S-Cartilage oligomeric protein (S-COMP), S-HA, S-CS846 epitope, S-type II collagen propeptide (S-PIICP) and U-type II collagen C telopeptide (CTX II/creatinin) were assayed. Variations over time for each biomarker were studied using Wilcoxon rank sum test.
Results: 45 patients (mean age 57.7, mean BMI 26.7) were analyzed. At baseline there was no difference between ITT and per-protocol population. Between D-15 and D1 there was no significant difference for any biomarkers (all $p>0.05$), indicating a good reproducibility in S and U measurements and the absence of spontaneous variation over time. At D1 WP was correlated with U-CTX II/creat (p=0.006). Between D1 and D90: Mean (SD) WP decreased from 57.7 (15.4) to 29.3(22.9) mm ($p<0.0001$). No variation was found for any S-biomarker. By contrast U-CTX II/creat was reduced by 20.5% and decreased significantly between D1 and D90 (385.1 vs 306.0 ng/mmol creat; p= 0.02). Furthermore U-CTX II and S-HA levels at baseline were both but independently predictive of clinical response to treatment (p=0.03 and p=0.02) even after adjustment for age, gender and BMI. Further more in logistic regression including age, BMI, bilaterality, KL grade, OA at other joints, DMOADS consumption and U-CTX II/creat at baseline, there was a significant correlation between clinical response and U-CTX II/creat level variation (p=0.03).
Conclusions: This study suggests that hylan IA injections are able to modify the knee joint metabolism in patients with OA resulting in a decrease in urine CTX II concentrations, particularly in patients with the highest levels of U-CTX II and HA before treatment. Further studies coupling biomarkers and imaging techniques are needed to investigate the possible chondroprotective effect of hylan in K-OA.

535

CHARACTERIZATION OF SUSTAINED RELEASE NATIVE AND MODIFIED HUMAN SFLT01 FORMULATION FOR INTRAARTICULAR DELIVERY TO TREAT OA PAIN

W. Cheng[1], J. Stefano[2], D. Li[2], J. Kyazike[2], K. Boudanova[2], H. Qiu[2], L. Hou[2], M. O'Callaghan[2], V. Kraus[1], G. Matthews[2]
[1]Duke Univ., Durham, NC; [2]Genzyme Corp., Framingham, MA

Purpose: VEGF is both a potent angiogenic and vascular permeability factor that is crucial in endochondral ossification involved in bone formation and remodeling. We hypothesize that osteoarthritis (OA) pain-associated bone marrow lesions, effusion, and synovitis are in part, driven by VEGF mediated increases in vascular permeability. Soluble fms-link tyrosine kinase 1 (sFlt1), a variant of VEGF receptor Flt1, can potentially block the function of VEGF. Our previous work demonstrated that VEGF inhibition using virally delivered sFlt decreased synovitis and pain marker expression in a rabbit OA model. However, the biological joint half life of recombinant human sFlt01, a VEGF neutralizing Fc fusion protein, was demonstrated to be just a few hours. In order to prolong the joint half life of sFlt01, we constructed a modified delivery form of this protein, verified its bioactivity, and confirmed enhanced stability compared to sFlt01 in accelerated stability tests up to 3 months.
Methods: Modified sFlt delivery construct TCEP reduction and CuCl2 oxidation were used to create a dimerized modified sFlt delivery construct, which was subsequently column purified and compared to native sFlt as well as the monomer forms of the modified delivery construct on silver stained SDS PAGE gels.
In vitro VEGF ELISA binding assay A two-fold serial dilution of sFlt01 or the modified sFlt delivery construct starting from 10,000 pM was made in assay medium (M199 media + 5% FBS + 1x Penn/Strep). VEGF165 (10 pM) was then incubated with the serial dilutions. Unbound VEGF for each dilution was measured by ELISA to identify whether any shift had occurred between VEGF neutralization by the modified sFlt delivery construct vs. native sFlt01 control.
Accelerated stability test Modified sFlt delivery construct (0.25 mg/ml) was incubated in PBS with 1X HALT protease inhibitor cocktail with EDTA (Pierce) at 45°C and aliquots drawn and flash frozen at -80°C. Unmodified sFlt01 at the same concentration was used as a control. The samples were collected over different time points in both 36 and 94 day stability tests. The samples with or without PNGase F treatment was further analyzed by silver-stained reducing SDS PAGE.
Results: TCEP reduction and CuCl2 oxidation resulted in successful dimerization of the modified sFlt delivery construct.
TCEP reduced protein monomer showed ~35 fold higher EC50 vs. native sFlt01 whereas Cu2+ oxidized dimer showed 2.2 - fold high EC50 compared to sFlt01.
From the accelerated stability test data, sFlt01 control showed increase amount of ~35 kDa product as well as high molecular weight of ~115 kDa product in silver stained reducing SDS PAGE without PNGase treatment. The modified sFlt delivery construct showed greater stability at elevated temperature, generating less high molecular weight aggregate and fragmentation compared to sFlt01 control at both 36 and 96 days.
Conclusion: The generated modified sFlt delivery construct expressed from HEK293 can be efficiently dimerized.
Copper-generated protein dimer retains high affinity for VEGF binding, comparable to native sFlt01.
The modified sFlt delivery construct appears more stable at elevated tem-